Neonatal Exposure of Male Rats to Nonylphenol Has No Effect on the Reproductive Tract

J. Odum and J. Ashby

Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ, UK

Received September 20, 1999; accepted April 17, 2000

To whom correspondence should be addressed. Fax: 44 0 1625 590249. E-mail: john.ashby@ctl.zeneca.com.

P. C. Lee (1998, Endocrine 9, 105–111) has reported that neonatal exposure of SD rats to nonylphenol (NP; 8 mg/kg/day) by daily intraperitoneal (ip) injection in DMSO results in decreased ventral prostate and epididymides weights, and delayed testes descent, at post natal day (pnd) 31. These effects were surprising given that similar effects were not reported in an earlier multi-generation study of NP. We have repeated the central experiment described by Lee and were unable to confirm the effects reported. Alpk (Wistar derived) rats were exposed to NP (8mg/kg/day by ip injection in either arachis oil or DMSO) from pnd 1–10 and assessed on pnd 34–36. No significant effects on animal body weights were observed. The weights of the epididymides, seminal vesicles, testes, and ventral prostate were unaffected using either vehicle. Testes descent proceeded normally, with both test and control testes fully descended by pnd 29. Possible reasons for this divergence in findings for NP are discussed.

Key Words: nonylphenol; male reproductive tract; testes; prostate.

In vitro and in vivo assays for estrogenicity. The minimum detection level for responses related to the estrogenic activity of NP in reproductive and mammary tissues is ~40 mg/kg/day. These levels are based on a dietary multi-generation study (US National Toxicology Program, 1997; Chapin et al., 1999), and uterotrophic and mammary gland assays where NP was given orally or via subcutaneous implants (Ashby et al., 1997; Odum et al. 1997, 1999a,b). However, intraperitoneal (ip) administration of NP appears to lower the minimum detection level of NP in the immature rat uterotrophic assay to 18 mg/kg (Lee and Lee, 1996). More recently, Lee (1998) reported that ip administration of NP to male rats at dose levels up to 8 mg/kg/day in the neonatal period caused reductions in the weights of male reproductive organs at postnatal day 31, and increased the frequency of cryptorchidism. The vehicle for these experiments was not stated, but it was DMSO (Lee 1998, personal communication), and the effects were inhibited by co-administration of the estrogen receptor (ER) antagonist, ICI 182,780.

A repeat study using the oral route of exposure gave negative results (Lee 1998, personal communication). Given that cryptorchidism was not observed in the earlier rat reproduction study of NP (US NTP, 1997; Chapin et al., 1999), it became of interest to confirm such a striking finding in an independent repeat study.

METHODS

Chemicals. NP was provided by Schenectady International and was from the same batch as that tested in a multi-generation study (US NTP, 1997; Chapin et al., 1999), a 90-day toxicity study (Cunny et al., 1997), and previous mammary gland and uterotrophic assays (Odum et al., 1999a,b).

Test protocols. The protocols for these studies were taken from the studies of Lee (1998), who dosed NP by ip injection at dose levels up to 8 mg/kg/day over a number of different neonatal time periods. The critical period of vulnerability to NP during male reproductive development was defined as the first 13 days of life. This conclusion was based on reductions in the weights of male reproductive organs observed when dosing commenced in the first 6 postnatal days, and not observed when dosing commenced at day 13 (illustrated for the ventral prostate weight) (Fig. 1). We carried out two studies using the highest dose of NP, exposed from postnatal days 1–10, used by Lee (1998). This period was selected because Lee (1998) had shown a >50% incidence of cryptorchidism with this protocol and had also described reductions in sex organ weights when NP was administered from postnatal days 1–5 or 1–15. Experiment 1 was a simple attempt to reproduce the primary findings of Lee (1998) using a control group and a group exposed to 8 mg/kg/day NP. Experiment 2 was an expanded study containing additional groups of NP co-administered with the estrogen receptor antagonist Faslodex (FAS) ( Wakeling et al., 1991). Arachis oil (5 ml/kg body weight) was used as vehicle in Experiment 1 because it is commonly used in endocrine disruption studies (Wakeling et al., 1991) and had been used in our earlier studies of NP (Odum et al., 1997, 1999a,b). In Experiment 2, NP was administered in DMSO (1 ml/kg) (Lee, personal communication) as in the original report. Experiment 2 also contained appropriate control groups for the FAS administration, including a group of rats administered NP and an additional dose of DMSO. The doses of FAS were administered by ip injection in DMSO (1 ml/kg) immediately after the administration of NP.

Alpk:APfSD (Alpk) strain of rats on day 1 of pregnancy (initial body weights 225–275g) were obtained from the AstraZeneca breeding unit (Alderley Park). The studies were performed in accordance with the UK “Animals (Scientific Procedures) Act”. Animal care and procedures were carried out according to in-house standards and as described previously (Odum et al., 1999a,b).

Two experiments were carried out. In both cases, the pregnant females were allowed to give birth normally (postpartum = d0) and male pups only were
dosed via ip from day 1 to day 10 postpartum. In Experiment 1, litters (4 per group) received NP (8 mg/kg/day) in arachis oil (5 ml/kg), and controls received arachis oil only (5 ml/kg). In Experiment 2, litters (5 per group) received the following doses: NP (8 mg/kg/day) in DMSO (1 ml/kg); NP (8 mg/kg/day) in DMSO (1 ml/kg) and an additional ip dose of DMSO (1 ml/kg); NP (8 mg/kg/day) in DMSO (1 ml/kg) and FAS i.p (0.5 mg/kg/day) in DMSO (1 ml/kg); and FAS ip only (0.5 mg/kg/day in DMSO). Controls received 1 ml/kg DMSO only. In both experiments, the female pups were retained with the litter, but not dosed, until weaning at day 28 when the females were culled. Testis descent was monitored by observation of the male pups from day 21 until all the testes were descended. In Experiment 1, all groups were complete by day 29. The slight difference between the experiments is probably due to natural variation or the animals in Experiment 2 being slightly heavier (Fig. 1) (Table 1).

In Experiments 1 and 2, NP had no effect on the weights of the male sex organs (testes, epididymides, seminal vesicles, and ventral prostate). Absolute tissue weights and terminal body weights were unaffected (Table 1), as were the adjusted weights (organ weight/100 g body weight) (Fig. 3). Liver and kidney weights were essentially unaffected in both experiments (data not shown).

A striking finding in Experiment 2 (DMSO as vehicle) was the presence of adhesions between the liver, peritoneum, and sometimes spleen or kidney in 40–68% of animals in the NP-dosed groups. These adhesions were not present in any animals not receiving NP or in Experiment 1. NP is a known irritant and has been shown to be a severe skin and eye irritant when evaluated in standard toxicity tests for irritation (Smyth

**RESULTS AND DISCUSSION**

NP had no effect on the growth rate of male pups in either experiment (Fig. 1). Pup survival was good (> 82%) in all cases. One dam from the control group in Experiment 1 produced only female pups and therefore the mother and pups were culled at birth, giving reduced numbers of litters in this group. Testes descended normally in all groups in both experiments (Fig. 2). In Experiment 2, testis descent was completed by day 26, whereas, in Experiment 1, both groups were complete by day 29. The slight difference between the experiments is probably due to natural variation or the animals in Experiment 2 being slightly heavier (Fig. 1) (Table 1).

In Experiments 1 and 2, NP had no effect on the weights of the male sex organs (testes, epididymides, seminal vesicles, and ventral prostate). Absolute tissue weights and terminal body weights were unaffected (Table 1), as were the adjusted weights (organ weight/100 g body weight) (Fig. 3). Liver and kidney weights were essentially unaffected in both experiments (data not shown).
et al., 1969). It is assumed that the adhesions resulted from NP-induced irritation at the site of the ip injection in DMSO. The use of arachis oil as vehicle in Experiment 1 probably protected against the irritant effect of NP. This local irritant effect of ip-injected NP could have had a direct (non-estrogenic) effect on the descent of the testes of the neonates, given that the testes reside in the abdominal cavity during postpartum days 1–10. Such a direct irritant effect may explain the effects on testes descent reported by Lee (1998), but no such effects were seen in the present studies.

**FIG. 2.** The effects of NP on testis descent in Experiments 1 and 2. Compounds were administered ip from day 1–10 postpartum (where birth = day 0). Data represent the cumulative percentage of animals with descended testes. In Experiment 1, compounds were administered in arachis oil: NP at 8 mg/kg/day (17 pups per group). Controls received arachis oil only (17 pups per group). In Experiment 2, compounds were administered in DMSO: NP only at 8 mg/kg/day (29 pups per group); NP at 8 mg/kg/day and DMSO (29 pups per group); NP at 8 mg/kg/day and FAS at 0.5 mg/kg/day (32 pups per group); and FAS only at 0.5 mg/kg/day (39 pups per group). Controls received DMSO only (35 pups per group).

**TABLE 1**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compounds Administered</th>
<th>Number analyzed</th>
<th>Terminal body weight (g)</th>
<th>Testis (g)</th>
<th>Epididymis (mg)</th>
<th>Seminal vesicles (mg)</th>
<th>Ventral prostate (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Arachis oil</td>
<td>17</td>
<td>99.2 ± 16.7</td>
<td>0.88 ± 0.20</td>
<td>86.6 ± 16.6</td>
<td>65.0 ± 10.4</td>
<td>56.9 ± 13.0</td>
</tr>
<tr>
<td>NP 8 mg/kg/day</td>
<td></td>
<td>17</td>
<td>105.5 ± 12.0</td>
<td>0.93 ± 0.15</td>
<td>95.6 ± 12.5</td>
<td>66.7 ± 11.9</td>
<td>57.5 ± 13.3</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>DMSO</td>
<td>25</td>
<td>134.8 ± 9.0</td>
<td>1.23 ± 0.09</td>
<td>132.2 ± 13.3</td>
<td>74.0 ± 8.0</td>
<td>69.3 ± 11.9</td>
</tr>
<tr>
<td>NP 8 mg/kg/day</td>
<td></td>
<td>25</td>
<td>134.1 ± 11.0</td>
<td>1.23 ± 0.16</td>
<td>129.4 ± 17.3</td>
<td>69.8 ± 10.1</td>
<td>64.1 ± 12.2</td>
</tr>
<tr>
<td>NP 8 mg/kg/day and DMSO</td>
<td></td>
<td>25</td>
<td>127.1 ± 7.6</td>
<td>1.16 ± 0.12</td>
<td>126.4 ± 12.8</td>
<td>70.2 ± 10.4</td>
<td>64.8 ± 15.2</td>
</tr>
<tr>
<td>FAS 0.5 mg/kg/day and NP 8 mg/kg/day</td>
<td></td>
<td>25</td>
<td>127.1 ± 12.7</td>
<td>1.19 ± 0.14</td>
<td>129.0 ± 14.7</td>
<td>67.3 ± 10.8</td>
<td>66.0 ± 11.1</td>
</tr>
<tr>
<td>FAS 0.5 mg/kg/day</td>
<td></td>
<td>25</td>
<td>129.6 ± 15.3</td>
<td>1.20 ± 0.14</td>
<td>128.9 ± 16.9</td>
<td>68.6 ± 10.0</td>
<td>64.8 ± 10.8</td>
</tr>
</tbody>
</table>

*Note.* Compounds were administered ip from day 1–10 postpartum (where birth = day 0). In Experiment 1, compounds were administered in arachis oil. In Experiment 2, compounds were administered in DMSO. Data are mean absolute weight ± SD.
We have been unable to reproduce the findings of Lee (1998) on cryptorchidism and changes in sex organ weights induced by NP. The reason for this is unclear. The only differences between our studies and those of Lee (1998) were that Lee used SD rats and terminated the studies at postnatal day 31. We used Alpk (Wistar derived) rats, and terminated the studies 3 to 5 days later. To date, SD rats have not been reported to be more sensitive than Alpk rats to estrogens. For example, NP has been shown to produce a similar uterotrophic effect in immature Alpk and SD rats (Odum et al., 1999b), and the SD rat was less sensitive than the F344 rat to the estrogenic effects of bisphenol A (Steinmetz et al., 1998; Long et al., 2000). The difference in termination dates is considered negligible for the endpoints being assessed. It is of interest that in a more recent report from the same group (Lee et al., 1999), where NP was administered to neonatal rats using an almost identical protocol (pnd 1–15, at 8 mg/kg/day, ip in DMSO), significant inter-animal variation was seen. Testicular atrophy and lowered sperm counts were observed in only 3 of 9 treated animals and cryptorchidism or changes in weights of the other male sex organs were not described.

Deficiencies in the design and conduct of the investigations reported by Lee (1998) and Lee et al. (1999) make it difficult to discuss further our inability to confirm those observations. Key deficiencies were as follows: ip injection is not an appropriate route of exposure for studies where the neonatal testes are under study; the use of DMSO as a vehicle for toxicity studies is questionable, given its membrane permeability and intrinsic toxicity; and in particular, ip injection of NP in DMSO provides data for the neonatal rat testes which is of little or no value.

**FIG. 3.** The effects of NP on the weight of male sex organs (g [testis] or mg [E, SV, VP]/100 g body weight). Compounds were administered ip from day 1–10 postpartum (where birth = day 0). Animals were killed on day 34-36. Data represent group means ± SD. In Experiment 1, compounds were administered in arachis oil: NP at 8 mg/kg/day. Controls received arachis oil only; n = 17 pups for all groups and determinations. In Experiment 2, compounds were administered in DMSO: NP only at 8 mg/kg/day; NP at 8 mg/kg/day and DMSO; NP at 8 mg/kg/day and FAS at 0.5 mg/kg/day; and FAS only at 0.5 mg/kg/day. Controls received DMSO only; n = 25 pups for all groups and determinations.
value for human or wildlife risk assessments of this agent. Faslodex was injected in DMSO into some of the animals previously injected with NP in DMSO, but the control animals received only a single injection of DMSO. The high variability of control prostate weights was not discussed (Fig. 4).

In the study by Lee et al. (1999), the handling of the testes weight data was not justified. Nine animals exposed to NP were segregated into subgroups of 3 and 6, with statistically significant effects only being seen among the 3 animals selected, compared to the total of 9 control animals. It is possible that only a sub-population of the animals were affected, but that cannot be confirmed by such arbitrary grouping of the animals. Further, in the analysis of testes weights, the right and left testes weights for these 3 animals, when compared to the total group of 6 control testes weights. Overall, these data do not provide adequate support for an estrogenic effect by NP on the testes of the neonatal rat.

REFERENCES


